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THE PROFILES OF WEEKLY PROGESTERONE AND ESTRADIOL CONCENTRATIONS DURING PREGNANCY IN EWES: THEIR CORRELATIONS WITH LAMB BIRTH WEIGHT

M.Y. Sumaryadi* & W. Manalu**

ABSTRACT

Thirty nine-pregnant ewes (20 and 19 carrying a single and multiple fetuses, respectively) were used to study the correlations of weekly maternal serum progesterone and estradiol concentrations during pregnancy with lamb birth weight at parturition. Blood samples were drawn weekly (weeks 0 to 20) during gestation period for determination of progesterone and estradiol concentrations. At parturition lamb birth weight was determined around 12 h post partum. Regardless of litter size, concentrations of progesterone during the fetal stage of pregnancy positively correlated with lamb birth weight. In the ewes carrying a single fetus, maternal serum progesterone at weeks 2, 4, 8, 10 to 12, 14, 16 and 18 to 20 of pregnancy positively correlated with lamb birth weight. In the ewes carrying multiple fetuses, maternal serum progesterone at weeks 0, 8, and 10 of pregnancy positively correlated with lamb birth weight. Regardless of litter size, concentrations of estradiol at weeks 0, 2, and 4 during the embryonal stage of pregnancy positively correlated with lamb birth weight. During the fetal stage of pregnancy, maternal serum estradiol concentrations positively correlated with lamb birth weight except at weeks 8, 9, and 20 of pregnancy. In the ewes carrying a single fetus, maternal serum estradiol at weeks 1, 2, 4, 6, 8, 10, 12, and 14 of pregnancy positively correlated with lamb birth weight. In the ewes carrying multiple fetuses, maternal serum estradiol concentrations at weeks 0, 1, 2, 4, 6, 8, 10, and 14 of pregnancy positively correlated with lamb birth weight.

Keywords: progesterone, estradiol, lamb birth weight, pregnancy, sheep

Progesterone and estradiol, along with other hormones directly related to pregnancy, are known for their roles in the maintenance of pregnancy through their effects on uterine stromal cell differentiation (Bell, 1984), secretion of uterine milk protein and stimulation of placental growth and hormone secretion during the embryonal stage of pregnancy (Bell, 1984; Wheeler *et al.*, 1987; Mulholland *et al.*, 1994). During the fetal stage of pregnancy (8 to 20 weeks of gestation), progesterone and placental lactogen are reported to influence nutrient flow to the placenta through their effects on the maternal tissue metabolism (Shirling *et al.*, 1981; Sutter-Dub *et al.*, 1981; Fowden, 1995).

Secretions of estradiol and progesterone increase dramatically with the changes in ovarian activity during the estrous cycle and pregnancy. In ovine, secretion of estradiol increases during proestrous along with the maturation of the follicle in the ovary, then decreases significantly during the embryonal stage of pregnancy, and increases precipitously during the fetal stage of pregnancy until parturition (Umo *et al.*, 1976; Pant *et al.*, 1977; Manalu *et al.*, 1996). Progesterone increases 2 days after ovulation, and it shows a marked rise from day 5 to a peak between days 7 and 13 (Umo *et al.*, 1976; Pant *et al.*, 1977). It remains almost stable during the first 7 weeks of pregnancy and increases dramatically after week 8 of pregnancy (Manalu *et al.*, 1996).

Previous observations suggest lower peripheral progesterone in overfed animals as a reason for the lower embryonal survival and birth weight (Hard and Anderson, 1979; Parr *et al.*, 1987; Ashworth, 1991). Supplementation of progesterone in the overfed animals during early pregnancy has been demonstrated to restore fetal growth and birth weight similar to those in the control animals (Kendall and Hays, 1960; Parr *et al.*, 1987; Ashworth, 1991). These results indicated that progesterone, and probably other hormones and factors secreted by the ovary, corpus luteum, placenta and uterus, could have significant roles in improving prenatal growth.

The dramatic changes in progesterone secretion with the increased litter size and the advance of pregnancy in sheep and goats could have physiological effects on prenatal growth. However, there are limited experiments to explore the possible correlation of the endogenous secretion of progesterone during pregnancy with prenatal growth in polytocous animals. This present study was designed to correlate weekly maternal serum progesterone and estradiol concentration during pregnancy with lamb birth weight at parturition in Javanese thin-tail ewes.

METHODS

Experimental Conditions and Animals

This experiment was conducted during the hot (25 to 32°C) and wet (70 to 80% relative humidity) season in the humid tropics of Indonesia. Experimental animals were 20 Javanese thin-tail ewes (5, 9, 4, and 2 ewes carrying 0, 1, 2, and 3 fetuses, respectively) with similar body weight (20 to 22 kg) and age (2 to3 years) at breeding. Javanese thin-tail sheep is a meat-type indigenous breed well recognized for its high prolificacy (Bradford *et al.*, 1986; Sutama *et al.*, 1988). The experimental ewes were injected twice with $PGF_{2\alpha}$ (i.m.) (Folligon, Intervet, North Holland, The Netherlands) at an 11-day interval. Three days after the last injection, the

* Laboratory of Physiology and Reproduction, Faculty of Animal Sciences, Jenderal Soedirman University, Purwokerto

** Department of Physiology and Pharmacology, Faculty of Veterinary Medicine, Bogor Agricultural University

experimental ewes were mated naturally by group breeding. At parturition, the weight of the lamb was determined around 12 h post partum.

Blood Sampling and Processing

Ten ml of blood samples were drawn with plain vacutainer or sterile syringes from the jugular vein between 0900 and 1000 h. The first blood samples were taken one day after the last prostaglandin injection (week 0 of pregnancy), and 10 days later (seven days after the predicted ovulation, as the end of week 1 of pregnancy). Additional blood samples were drawn weekly on Thursday until parturition. Blood samples were allowed to clot in a cool ice box, centrifuged to separate serum (2500 rpm for 30 minutes), which was then frozen for estradiol and progesterone analyses.

Progesterone and Estradiol Analyses

The concentration of serum progesterone was measured in duplicate by solid-phase radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA) using I^{125}-progesterone as a tracer, with a slight modification to accommodate wide ranges of progesterone concentrations in pregnant ovine (Manalu *et al.*, 1996). The progesterone assay used the whole serum without prior ether extraction. The radioactivities of I^{125}-progesterone-bound tubes were counted with an automatic gamma counter. The lowest and highest limits of sensitivity of assay were 0.1 and 20 ng/ml, respectively. Therefore, the concentrations of standard progesterone used to construct the standard curve ranged from 0.1 to 20 ng/ml. A sample volume of 100 µl serum was used in the assay of samples with progesterone concentrations ranged from 0.1 to 20 ng/ml. For samples with progesterone concentrations lower than 0.1 ng/ml, sample volume was increased to 200 µl. For samples with progesterone concentrations higher than 20 ng/ml, sample volume was reduced to 50 µl. Inter- and intra-assay coefficients of variation were 6, and 4%, respectively. Concentrations of progesterone were parallel in the sample volumes of 50, 100, and 200 µl.

The concentration of serum 17-β estradiol was measured in duplicate by the solid-phase technique radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA) using I^{125}-estradiol as a tracer, with a slight modification to accommodate wide ranges of estradiol concentrations in pregnant ovine (Manalu *et al.*, 1996). The estradiol assay used the whole serum without prior ether extraction. The radioactivities of I^{125}-estradiol-bound tubes were counted with an automatic gamma counter. The lowest and highest limits of sensitivity of assay were 20 and 150 pg/ml, respectively. Therefore, the concentrations of standard estradiol used to construct the standard curve ranged from 20 to 150 pg/ml. A sample volume of 100 µl serum was used in the assay for samples with estradiol concentrations ranged from 20 to 150 pg/ml. For samples with estradiol concentrations lower than 20 pg/ml, sample volume was increased to 200 to 300 µl to bring the estradiol concentrations to the range of standard used. For samples with estradiol concentrations higher than 150 pg/ml, sample volume was decreased to 50 µl to bring the estradiol concentrations to the range of standard used. All samples' estradiol concentrations were within the range of concentrations of standard estradiol used to construct the standard curve. Inter- and intra-assay variations coefficients were 7 and 5.0%, respectively. The concentrations of estradiol were parallel in the sample volumes of 50, 100, 200 and 300 µl.

Statistical Analyses

Weekly estradiol and progesterone concentrations during pregnancy were regressed with mammary dry-fat free tissue (DFFT), deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and collagen at parturition using simple regression and correlation analyses (Neter *et al.*, 1985).

RESULTS

Weekly concentrations of maternal serum progesterone during pregnancy in the experimental ewes are presented in Fig. 1. During the first 8 weeks of pregnancy, maternal serum progesterone concentration slowly increased, it rise rapidly and reached peak concentrations around weeks 12 to 17 of pregnancy, and it decreased near parturition. In the ewes carrying multiple fetuses, concentrations of progesterone were significantly higher than in those carrying a single fetus. The highest difference in maternal serum progesterone between the ewes carrying a single and multiple fetuses occurred during weeks 12 to 17 of pregnancy.

Figure 1. Weekly serum progesterone concentrations during pregnancy in the nonpregnant ewes (◆), ewes carrying a single (▲) and multiple (■) fetuses in Javanese thin-tail sheep.

The profile of weekly estradiol concentrations in the maternal serum during pregnancy in the experimental ewes is presented in Fig. 2. The concentrations of estradiol were constantly low during the first 8 weeks of pregnancy. After week 8 of pregnancy, estradiol concentration in the pregnant ewes constantly increased until parturition, and being higher in the higher litter size. In the ewes carrying multiple fetuses, the concentrations of estradiol were significantly higher than in those carrying a single fetus. The greatest differences in maternal serum estradiol concentrations between the ewes carrying a single and multiple fetuses occurred during weeks 12 to 20 of pregnancy.

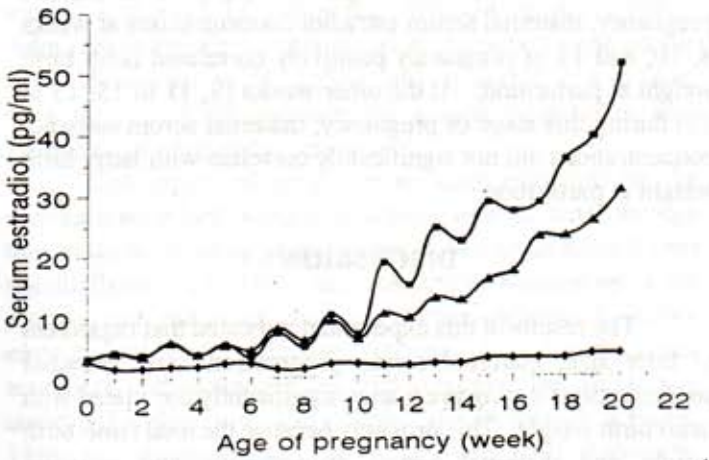

Figure 2. Weekly serum estradiol concentrations during pregnancy in the nonpregnant ewes (◆), ewes carrying a single (▲) and multiple (■) fetuses in Javanese thin-tail sheep.

The increased litter size increased the total lamb birth weight curvelinearly, with a resultant decreased in average lamb birth weight ($P < 0.01$), as is normally observed in prolific ruminants. Total lamb birth weights in the ewes carrying 1, 2, or 3 fetuses were 1.9 ± 0.1, 3.2 ± 0.2, and 4.2 ± 0.4 kg, respectively. Total lamb birth weight in the ewes carrying triplets increased by 114 and 28% when compared to those carrying a single or twin fetuses, respectively. The ewes carrying twin fetuses had a total lamb birth weight increase by 67% when compared to those carrying a single fetus. The mean lamb birth weight in the ewes carrying triplet fetuses decreased by 28 and 17% when compared to those in carrying a single or twin fetuses, respectively. In the ewes carrying twin fetuses the average lamb birth weight decreased by 17% when compared to those carrying a single fetus ($P < 0.05$).

Correlations of weekly maternal serum progesterone concentrations during pregnancy with lamb birth weight at parturition are presented in Table 1. Regardless of litter size, the concentrations of maternal serum progesterone at weeks 0 and 6 during the embryonal stage (weeks 0 to 7) of pregnancy positively correlated with the total lamb birth weight at parturition. At the other weeks during this stage of pregnancy, maternal serum progesterone concentrations did not significantly correlate with lamb birth weight. During the fetal stage (weeks 8 to 20) of pregnancy, the concentrations of maternal serum progesterone positively correlated with the total lamb birth weight at parturition. Higher maternal serum progesterone concentrations during the fetal stage of pregnancy associated with the higher total lamb birth weight at parturition.

Table 1. Coefficients of correlation between weekly maternal serum progesterone concentrations during pregnancy and lamb birth weight at parturition in Javanese thin-tail ewes.

Weeks of pregnancy	Litter size		
	Total	Singleton	Multiple
0	0.40**	0.19	0.53*
1	0.18	0.05	0.00
2	0.11	0.66**	0.19
3	0.21	0.24	0.22
4	0.07	0.47*	0.27
5	0.06	0.33	0.07
6	0.39**	0.28	0.38
7	0.29	0.29	0.00
8	0.84**	0.72**	0.82**
9	0.54**	0.03	0.33
10	0.63**	0.47*	0.68**
11	0.58**	0.40*	0.23
12	0.47**	0.40*	0.01
13	0.52**	0.36	0.12
14	0.49**	0.70**	0.38
15	0.53**	0.16	0.44
16	0.62**	0.46*	0.38
17	0.56**	0.25	0.34
18	0.62**	0.66**	0.29
19	0.53**	0.46*	0.31
20	0.35*	0.52*	0.28

*P < 0.05
**P < 0.01

In the ewes carrying a single fetus, serum progesterone concentrations at weeks 2 and 4 during the embryonal stage of pregnancy positively correlated with lamb birth weight. Maternal serum progesterone concentrations at other weeks during this stage of pregnancy had lower correlation with lamb birth weight. During the fetal stage of pregnancy, maternal serum progesterone concentrations at weeks 8, 10, 11, 12, 14, 16, 18, 19, and 20 of pregnancy positively correlated with lamb birth weight at parturition. In this stage of pregnancy, maternal serum progesterone at weeks 9, 13, 15, and 17 of pregnancy had low correlations with lamb birth weight.

In the ewes carrying multiple fetuses, maternal serum progesterone concentrations at weeks 0 during the embryonal stage of pregnancy positively correlated with lamb birth weight at parturition. At the other weeks during this stage of pregnancy (weeks 1 to 7) maternal serum progesterone had low correlation with lamb birth weight. During the fetal stage of pregnancy, maternal serum progesterone concentrations at weeks 8 and 10 had positive correlation with lamb birth weight at parturition. At the other weeks during this stage of pregnancy (weeks 9, 11 to 20), maternal serum progesterone concentrations did not correlate with lamb birth weight at parturition.

Correlation of weekly maternal serum estradiol concentrations during pregnancy with lamb birth weight are presented in Table 2. Regardless of litter size, the concentrations of maternal serum estradiol at weeks 0, 2, and 4 during the embryonal stage of pregnancy positively correlated with the total lamb birth weight at parturition. At the other weeks during this stage (weeks 1, 3, 5, 6, and 7) of pregnancy, maternal serum estradiol concentrations had low correlations with lamb birth weight. At weeks 10 to 19 during the fetal stage of pregnancy, the concentrations of maternal serum estradiol positively correlated with the total lamb birth weight at parturition. At the other weeks (8, 9, and 20) of pregnancy, maternal serum estradiol concentrations had low correlations with lamb birth weight. In general, higher maternal serum estradiol concentrations during the fetal stage of pregnancy associated with the higher total lamb birth weight at parturition.

Table 2. Coefficients of correlation between weekly maternal serum estradiol concentrations during pregnancy and lamb birth weight at parturition in Javanese thin-tail ewes.

Weeks of pregnancy	Litter size		
	Total	Singleton	Multiple
0	0.42**	0.02	0.72**
1	0.23	0.63**	0.49*
2	0.41**	0.63**	0.66**
3	0.10	0.03	0.18
4	0.40**	0.60**	0.60**
5	0.02	0.16	0.02
6	0.21	0.43*	0.65**
7	0.05	0.16	0.18
8	0.23	0.69**	0.55**
9	0.13	0.14	0.37
10	0.55**	0.56**	0.70**
11	0.30*	0.16	0.16
12	0.58**	0.64**	0.25
13	0.38*	0.16	0.03
14	0.67**	0.48*	0.46*
15	0.53**	0.03	0.40
16	0.43**	0.23	0.13
17	0.33*	0.04	0.34
18	0.38*	0.29	0.13
19	0.38*	0.18	0.28
20	0.28	0.03	0.02

*P < 0.05
**P < 0.01

In the ewes carrying a single fetus, serum estradiol concentrations at weeks 1, 2, 4, and 6 during the embryonal stage of pregnancy positively correlated with lamb birth weight. At the other weeks (0, 3, 5, and 7) during this stage of pregnancy, maternal serum estradiol concentrations did not significantly correlate with lamb birth weight. During the fetal stage of pregnancy, maternal serum estradiol concentrations at weeks 8, 10, 12, and 14 of pregnancy positively correlated with lamb birth weight at parturition. At the other weeks (9, 11, 13, and 15 to 20) during this stage of pregnancy, maternal serum estradiol concentrations had low correlation with lamb birth weight.

In the ewes carrying multiple fetuses, maternal serum estradiol concentrations at weeks 0, 1, 2, 4, and 6 during the embryonal stage of pregnancy positively correlated with lamb birth weight. At the other weeks (3, 5, and 7) during this stage of pregnancy, maternal serum estradiol concentrations had low correlation with lamb birth weight. During the fetal stage of pregnancy, maternal serum estradiol concentrations at weeks 8, 10, and 14 of pregnancy positively correlated lamb birth weight at parturition. At the other weeks (9, 11 to 13, 15 to 20) during this stage of pregnancy, maternal serum estradiol concentrations did not significantly correlate with lamb birth weight at parturition.

DISCUSSION

The results of this experiment indicated that regardless of litter size, maternal serum progesterone and estradiol concentrations during pregnancy significantly correlated with lamb birth weight. This probably because the total lamb birth weight and maternal serum progesterone and estradiol concentrations during pregnancy increased with the increased litter size (Sumaryadi & Manalu, 1999). When litter size was separated, however, the results were different for respective litter size.

In the ewes carrying a single fetus, maternal serum progesterone and estradiol concentrations significantly correlated with lamb birth weight at certain weeks throughout pregnancy. The nonsignificant correlations at other weeks probably due to the weekly fluctuation of the progesterone and estradiol concentrations (Fig. 1 and Fig. 2). Mean serum progesterone concentrations during the fetal stage of pregnancy (weeks 8 to 20) in the ewes carrying a single fetus positively correlate with lamb birth weight (Manalu & Sumaryadi, 1999), and ewes with the higher estradiol concentrations at any progesterone concentration measured had heavier lamb birth weight (Manalu & Sumaryadi, 1998). The results of the present study indicated that in the ewes carrying a single fetus, the higher the serum progesterone and estradiol concentrations throughout pregnancy the heavier the lamb birth weight at parturition.

In the ewes carrying multiple fetuses, maternal serum progesterone and estradiol concentrations at the beginning and at mid pregnancy had positive correlations with lamb birth weight. At the other weeks of pregnancy, the correlations were still positive, but were not statistically significant. The results

indicated that in the ewes carrying multiple fetuses, the higher the serum progesterone and estradiol concentrations the heavier the lamb birth weight. Mean serum progesterone and concentrations during the fetal stage of pregnancy (weeks 8 to 20) in the ewes carrying multiple fetuses positively correlate with lamb birth weight (Manalu & Sumaryadi, 1999), and ewes with the higher estradiol concentrations at any progesterone concentration measured had heavier lamb birth weight (Manalu & Sumaryadi, 1998). The results of the present study indicated that in the ewes carrying multiple fetuses, the higher the serum progesterone and estradiol concentrations throughout pregnancy the heavier the lamb birth weight at parturition.

The results of the experiment suggested that higher maternal serum progesterone during pregnancy could improve prenatal growth and lamb birth weight. Superovulated ewes having higher serum progesterone concentrations throughout pregnancy had heavier individual fetal weight measured at weeks 7 and 15 of pregnancy (Manalu *et al.*, 1998). Administrations of progesterone on days 1 to 6 of pregnancy in sheep (Kleemann *et al.*, 1994), and on days 1 to 4 in cows (Garrett *et al.*, 1988) enhance fetal growth measured on days 74 and 14 of pregnancy, respectively.

The effect of progesterone and estradiol on the increased lamb birth weight could have been mediated through their roles in directing gene expression in uterine stromal cells (Mulholland *et al.*, 1994) that resulted in stimulation of the uterine and placental growth. Better developed uterine environment could increase nutrients and growth factor secretions and exchanges required to support the developing embryo (Findlay *et al.*, 1981; Bell, 1984; Ashworth & Bazer, 1989a,b; Ashworth *et al.*, 1989; Ashworth, 1991; Ashworth, 1992). Current results indicated that superovulated ewes with higher serum progesterone concentration had heavier uterine tissue and fetal weight at the end of the embryonic stage of pregnancy (week 7 of pregnancy) (Manalu *et al.*, 1998).

Better developed placentas could contribute more to the growth of the fetus (Robinson *et al.*, 1995), as nutrients supply in the placenta was not a limiting factor. The effect of progesterone and estradiol concentrations during the fetal stage of pregnancy on lamb birth weight is probably, in part, mediated by the reported effects of progesterone on body energy reserves partitioning to the placenta. Some reports indicated that higher progesterone levels could mobilize more fatty acid and glucose from body reserves to the maternal circulation (Shirling *et al.*, 1981; Sutter-Dub *et al.*, 1981; Fowden, 1995) that in turn could be used by the fetus through the placenta and increased fetal growth (Robinson *et al.*, 1995; Fowden, 1995). A better developed placenta produced more placental lactogen and other hormones and factors (Robinson *et al.*, 1995) that could eventually influence fetal growth through their effects on maternal body energy reserves mobilization (Annison *et al.*, 1984; Bell, 1984; Fowden, 1995) and fetal glycogen synthesis (Hill, 1989; Breier *et al.*, 1994).

Growth and development of the uterine tissues with the overall biochemical changes before implantation are initiated by estradiol secreted during preovulation, which are then continued by progesterone (Mulholland *et al.*, 1994) and probably by other hormones and growth factors secreted by the corpus luteum during the luteal phase of the estrous cycle. Growth and development of the fetus during the fetal stage of pregnancy are affected by the growth and development of the placenta (Robinson *et al.*, 1995), nutrients availabilities in the maternal, placental and the fetal circulations (Harding & Johnston, 1995; Hay, 1995), and the endocrine status of the mother and the fetus (Fowden, 1995).

CONCLUSION

The results of this experiment indicated that the maternal concentrations of progesterone and estradiol throughout pregnancy had positive correlation with lamb birth weight. The results suggested that increasing maternal serum progesterone and estradiol during pregnancy, either by exogenous administration or by increasing their endogenous secretion by superovulation, might be used to improve prenatal growth and lamb birth weight.

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